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REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-33 are in this case. Claims 1-17, and 29-33 have been withdrawn from consideration. Claims 18-28 have been rejected. Claims 18, 20-22, 24-26 and 28 have now been amended. Claim 19 has been cancelled.

35 U.S.C. § 112, First paragraph Rejections

The Examiner has rejected claims 18, 23 and 25-27 under 35 U.S.C. 112, first paragraph, for lack of enablement. The Examiner's rejections are respectfully traversed. Claims 18 and 24-26 have now been amended.

The Examiner has stated that the specification, while being enabling for repopulating a devitalized acellular three dimensional scaffold with pluripotent stem cells, progenitor cells derived from the same tissue source as the scaffold, and/or differentiated cells originating from the same tissue type as the scaffold, does not reasonably provide enablement for repopulating the scaffold with any differentiated cell type, and as such does not enable any person skilled in the art to make and/or use the invention commensurate in scope with claims 18, 23 and 25-27. The Examiner has recommended that claims should be narrowed to encompass only either pluripotent stem cells or differentiated cells from the organ from which the scaffold originates.

In accordance with the Examiner's recommendation, and in order to further expedite this case, independent claim 18 has now been amended to recite:

18. A method of generating an artificial micro-organ comprising:
- (a) providing devitalized, acellular, tissue-derived three dimensional scaffold, said acellular three dimensional scaffold being of dimensions selected such that when populated with cells, said cells ~~positionable~~ deepest within said scaffold are at least about 100 micrometers and not more than about 225 micrometers away from said cells positioned at a nearest surface exposed to a source of gas and nutrients formed on said scaffold; and
 - (b) seeding said acellular three dimensional scaffold with stem cells, progenitor cells or homologous differentiated cells, and

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(c) providing conditions for cell growth and proliferation.

Thus, now amended claim 18, and all claims dependent therefrom, read on scaffolds comprising acellular, tissue-derived micro organs seeded with stem, progenitor or homologous differentiated cells. Support for such an amendment is found throughout the instant specification, for example, in claims 19 (now cancelled), 22 and 28, and page 24, lines 17-19:

“...such a scaffold when seeded with stem cells (adult or embryonic) and optionally differentiated cells can be used to generate a micro-organ like three dimensional tissue structure.”

And page 41, lines 9-11:

“Taken together, these results demonstrate the ability of micro organs to support the growth and proliferation of embryonic and adult derived stem cells, of homologous and non-homologous tissue and species origin.”

In view of the abovementioned arguments and amendments, Applicant believes to have overcome the 112, first paragraph rejections.

35 U.S.C. § 102 Rejections –Vacanti (1999, US Patent No. 5,855,610), Vacanti (1998, US Patent No. 5,770,417) and Riviere (1995, PNAS, 92:6733-37)

The Examiner has rejected claims 18-28 under 35 USC § 102(b) as being anticipated by Vacanti (1999, US Patent No. 5,855,610) (Vacanti I) and claim 18 as being anticipated by Vacanti (1998, US Patent No. 5,770,417)(Vacanti II). The Examiner's rejections are respectfully traversed. Claims 18, and and 28 have now been amended.

In the telephone interview conducted with regard to this case on April 12, 2005, the definition of devitalized, acellular vs synthetic scaffolds was clarified, and it was made clear that the inclusion of the terms “devitalized, acellular” in claim 18 is directed to scaffolds formed from tissue-derived micro-organs from which cellular material has been removed, and that the term excludes synthetic scaffolds derived from non-living materials (see interview summary dated April 19, 2005).

In order to further clarify the non-synthetic character of the scaffolds formed from micro-organs of the present invention, Applicant has elected to include the additional limitation of “tissue derived” scaffolds in independent claim 18, and

dependent claims 25 and 26:

"Claim 18. A method of generating an artificial micro-organ comprising:

(a) providing devitalized, acellular, tissue-derived three dimensional scaffold..."

Claim 25. The method of claim 18, further comprising the step of generating said acellular three dimensional scaffold from a tissue-derived micro-organ.

Claim 26. The method of claim 25, wherein said step of generating is effected by subjecting said tissue-derived micro-organ to conditions selected suitable for removing cells and not acellular matrix from said micro-organ.

Thus amended claims 18, 25 and 26 now clearly exclude synthetic or other scaffolds not originating from living tissue. As such, it is Applicant's strong opinion that the synthetic constructs taught by Vacanti I and Vacanti II do not anticipate nor render obvious the methods of generating artificial microorgans of the present invention as now claimed.

The Examiner has further rejected claims 18-20 and 22-28 under 35 USC 102(b) as being anticipated by Riviere (1995, PNAS, 92:6733-37). The Examiner's rejections are respectfully traversed. Claims 18, 20-22, 24-26 and 28 have now been amended. Claim 19 has been cancelled, rendering moot the rejection thereof.

The Examiner has stated that Riviere teaches transplanting bone marrow cells into lethally irradiated mice, and that the lethal irradiation produces, within the medullary space, an acellular scaffold comprising extracellular matrix. Applicant strongly disagrees with Examiner's contention.

In this regard, the Examiner has asserted that devitalized, acellular scaffolds made from tissue explants are not completely devoid of cells, citing Mitchell (US 2002/0115208). It is Applicant's strong belief that the Examiner has misinterpreted the cited prior art.

Mitchell teaches a range of degrees of decellularization of tissue matrices, from partial to complete decellularization, using a variety of decellularization techniques. This is evident from the cited paragraph:

"[0111] The examples of decellularization techniques

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provided above are not intended to be limiting, and the invention encompasses the use of essentially any decellularization technique that removes a substantial fraction of the cells while leaving the matrix substantially intact...One of ordinary skill in the art will be able to select an appropriate decellularization technique and to vary parameters such as temperature and time in order to achieve a desired degree of decellularization. In certain embodiments of the invention the decellularization process removes at least 50% of the cells. In certain embodiments of the invention the decellularization process removes at least 60%... at least 90%, at least 95%, or substantially all of the cells are removed. As described above, there may be a tradeoff between the two goals of achieving a high degree of decellularization and preserving the structure and properties of the extracellular matrix. Thus it is not necessarily preferred to achieve maximal possible decellularization if doing so results in unacceptable damage to the extracellular matrix. The optimum degree of decellularization may depend upon the properties of the construct and the use for which it is intended."

Thus, Mitchell teaches that while maximum decellularization is achievable, it is not necessarily desirable. Further, in a later paragraph describing methods of preservation of decellularized matrices, Mitchell teaches decellularization by lyophilization and air drying techniques, some of which are known to cause 100% cell death in mammalian cells:

"[0125] Although cryopreservation represents a reliable approach to storing a decellularized tissue engineered construct of the present invention, alternative methods are also within the scope of the invention. For example, drying methods can also be used, with the addition of stabilizing compounds such as sucrose. A dextran and sucrose combination provides desirable physical properties and protein protection against freeze drying and air drying stresses. Freeze drying may take place using a lyophilizer. Air drying may take place under a stream of dry nitrogen, and the construct may then be lyophilized under a vacuum at room temperature.

Thus, in contrast to the Examiner's assertion, it is Applicants strong opinion that Mitchell teaches that devitalized scaffolds, if desired, can be completely void of cells.

More importantly, Applicant wishes to point out that the instant specification teaches that micro organs from non-resistant animal tissue, when co-cultured with neomycin-resistant embryoid bodies and embryonic stem cells, in the presence of neomycin for greater than 21 days, lose all signs of cellular vitality, but efficiently support the proliferation of the resistant embryoid bodies and stem cells (see Examples and Figures 4, 5 and 6 of the instant specification).

Thus, the "acellular" devitalized scaffolds of the present invention are, by definition, devoid of cells. Thus, while the lethally irradiated bone of Riviere et al. is partially decellularized, it is neither acellular nor devitalized, and as such the method of lethal irradiation and bone marrow transplantation of Riviere does not, and cannot anticipate the method of the present invention, as taught in claims 18-20 and 22-28.

35 U.S.C. § 103(a) Rejections – Mitchell (US2002/0115208, August 2002) or Bruchman (US Patent No. 5,879,383, March 1999), in view of Vacanti (1999, US Patent No. 5,855,610) or Vacanti (1998, US Patent No. 5,770,417)

The Examiner has rejected claims 18-24 under 35 USC § 103(a) as being unpatentable over Mitchell (US2002/0115208, August 2002), or Bruchman (US patent No. 5,879,383) in view of Vacanti (1999, US Patent No. 5,855,610)(Vacanti I) and Vacanti (1998, US Patent No. 5,770,417)(Vacanti II). The Examiner's rejections are respectfully traversed. Claims 18, 20-22, 24-26 and 28 have now been amended. Claim 19 has been cancelled, rendering moot the rejection thereof.

As reported in the interview summary (dated April 19, 2005), the Examiner has stated that the prior art of Bruchman and Mitchell anticipates the present invention as claimed as the physical limits of diffusion would prevent cells from populating the deepest parts of thicker tissue explants taught by Mitchell and by Bruchman. Applicant wishes to point out that it is precisely this limitation which has been addressed by the teachings of the present invention. By carefully choosing dimensions of the tissue explants to be used to make microorgans and scaffolds of the present invention, the present inventor has succeeded in remarkably enhancing the long term viability of cellular microorgans (see US Patent No. 5,888,720 to Mitrani, issued March 30, 1999), and the efficiency of repopulation of devitalized,

acellular tissue-derived micro organs (see Figs. 4, 5 and 6 of the instant specification).

Seeding conventional acellular matrices with cells for repopulation has, as noted by the Examiner (see Office Action, page 8, second paragraph), traditionally resulted in initial growth, followed by inevitable dying-back of the innermost cells, as cell growth increases towards desirable levels of cell density. This is due to the eventual limitation of nutrient, waste and gas exchange across the porous scaffolds by the cells at the surface most closely in contact with the nutrient medium:

"In tissue engineering, a highly porous artificial extracellular matrix or scaffold is required to accommodate mammalian cells and guide their growth and tissue regeneration in three dimensions. However, existing three-dimensional scaffolds for tissue engineering proved less than ideal for actual applications, not only because they lack mechanical strength, but they also do not guarantee interconnected channels." (Yang et al, Tissue Eng 2001; 7:679-89)

For example, in a recent study Li et al reported significant limitations to cell growth and proliferation with widely used Gelfoam scaffolds:

cells delivered by means of injection in a single bolus into the middle of a block of Gelfoam mesh preferentially migrated over the course of a week to the uppermost surface of the patch. All cell types examined formed a layer on this uppermost surface... The depth of this cell layer may have been due to the limitation of passive diffusion through the culture medium for delivery of nutrients and removal of metabolites from these cells. This might also account for the necrosis of a significant fraction of the cells injected in one bolus into the center of the Gelfoam patch. (Li et al J of Thor and Cardiovas Surg 2000;119: 368-375).

Li et al reported that cells grown on the Gelfoam scaffolds were viable no longer than 14 days, and that no angiogenesis was observed after engraftment. In stark contrast, cell proliferation and growth in microorgans of the present invention exceeds 21 days (see, for example, U.S. Patent No. 5,888,720) and vascular formation around implanted microorgans of the present invention has been repeatedly demonstrated (see, for example, instant Fig. 12).

Further, the advantages of tissue-derived over synthetic scaffolds have long been evident: The micro-architecture of natural tissue differs significantly from the synthetic polymers, foams and gels commonly comprising synthetic scaffolds, and the natural combinations of tissue-derived bioactive molecules (such as ECM and other cell-signaling molecules) persist after decellularization of the macro organs, overcoming the need to artificially add important bioactive molecules in synthetic scaffolds, providing superior adhesion and interaction with the seeded cells:

“Cells are able to detect very sensitively the mechanical properties of the adhesion substrate and regulate the integrin binding, assembly of focal adhesion plaques and cytoskeleton accordingly. If the adhesion substrate is very firm, rigid and non-deformable, for example ECM molecules adsorbed on too hydrophobic surfaces, the cells are not able to reorganize these molecules in order to access the ligands for integrin receptors and recruit these receptors into focal adhesion plaques, which is a prerequisite for delivery of signals ensuring the viability of anchorage-dependent cells. On the other hand, if the material is too elastic, compliant, flexible and irreversibly deformable, it does not allow the anchorage of cells, even if the ligands for integrin receptors are present in satisfactory amounts and accessibility, and are bound by these receptors. Such type of substrate cannot resist the cell tractional forces generated by the assembling cytoskeleton.”(Bacakova et al, Physiol Res 2004;53 (Suppl 1) S35-45)

Thus, the artificial micro organs generated from acellular, devitalized tissue-derived scaffolds of the present invention, provide a solution to the long felt need for scaffolds of natural origin, for cell implantation and/or tissue engineering, which can accommodate diffusion of nutrients and vital populations of cells throughout the entire thickness of the micro organ, without the limitations of growth inhibition and necrosis of the most deeply located cells.

With regard to the dimensions of the micro organs of the present invention, Examiner has indicated, during the interview, that, in order to more clearly distinguish the micro organs and scaffolds of present invention from the prior art tissue segments, the thickness of the micro organs and scaffolds may be defined by the maximal depth at which any cell is positionable in the scaffold, rather than reciting the depth at which any cell is positioned. Thus, independent claim 18 has now been amended to recite

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the limitation of a positionable depth of about 100-225 micrometers, or a maximal thickness of about 200-450 micrometers in the thinnest plane:

"...said acellular three dimensional scaffold being of dimensions selected such that when populated with cells, said cells positionable deepest within said scaffold are at least about 100 micrometers and not more than about 225 micrometers away from said cells positioned at a nearest surface exposed to a source of gas and nutrients formed on said scaffold..."

Thus, amended claim 18, and all claims directly or indirectly dependent therefrom, teach the preparation of micro organs from scaffolds having no greater than about 450 microns thickness in their thinnest plane, such that the deepest possible depth within the scaffold at which a cell is positionable is no greater than about 225 micrometers from the nearest source of gas and nutrients, clearly distinguishing the micro organs of the present invention from the thicker tissue portions taught by Mitchell and Bruchman. Support for such an amendment is found throughout the instant specification, for example, claims 4, 10, 16 and 36 (withdrawn).

In view of the above amendments and remarks it is respectfully submitted that amended claims 18, 20-22, 24-26 and 28, and all claims which directly or indirectly depend therefrom are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,



Sol Sheinbein

Registration No. 25,457

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